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Discovery of sarolaner: A novel, orally administered, broad-spectrum, isoxazoline ectoparasiticide for dogs

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ABSTRACT

The novel isoxazoline ectoparasiticide, sarolaner, was identified during a lead optimization program for an orally-active compound with efficacy against fleas and ticks on dogs. The aim of the discovery program was to identify a novel isoxazoline specifically for use in companion animals, beginning with *de novo* synthesis in the Zoetis research laboratories. The sarolaner molecule has unique structural features important for its potency and pharmacokinetic (PK) properties, including spiroazetidine and sulfone moieties. The flea and tick activity resides in the chirally pure S-enantiomer, which was purified to alleviate potential off-target effects from the inactive enantiomer. The mechanism of action was established in electrophysiology assays using CHO-K1 cell lines stably expressing cat flea (*Ctenocephalides felis*) RDL (resistance-to-dieldrin) genes for assessment of GABA-gated chloride channel (GABACls) pharmacology. As expected, sarolaner inhibited GABA-elicited currents at both susceptible (CfRDL-A285) and resistant (CfRDL-S285) flea GABACls with similar potency. Initial whole organism screening was conducted *in vitro* using a blood feeding assay against *C. felis*. Compounds which demonstrated robust activity in the flea feed assay were subsequently tested in an *in vitro* ingestion assay against the soft tick, *Ornithodoros turicata*. Efficacious compounds which were confirmed safe in rodents at doses up to 30 mg/kg were progressed to safety, PK and efficacy studies in dogs. *In vitro* sarolaner demonstrated an LC_{80} of 0.3 μg/mL against *C. felis* and an LC_{100} of 0.003 μg/mL against *O. turicata*. In a head-to-head comparative *in vitro* assay with both afoxolaner and fluralaner, sarolaner demonstrated superior flea and tick potency. In exploratory safety studies in dogs, sarolaner demonstrated safety in dogs ≥ 8 weeks of age upon repeated monthly dosing at up to 20 mg/kg. Sarolaner was rapidly and well absorbed following oral dosing. Time to maximum plasma concentration occurred within the first day post-dose. Bioavailability for sarolaner was calculated at >85% and the compound was highly protein bound (>99.9%). The half-life for sarolaner was calculated at 11–12 days. Sarolaner plasma concentrations indicated dose proportionality over the range 1.25–5 mg/kg, and these same doses provided robust efficacy (> 99%) for ≥35 days against both fleas (*C. felis*) and multiple species of ticks (*Rhipicephalus sanguineus*, *Ixodes ricinus* and *Dermacentor reticulatus*) after oral administration to dogs. As a result of these exploratory investigations, sarolaner was progressed for development as an oral monthly dose for treatment and control of fleas and ticks on dogs.

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1. Introduction

In spite of an abundance of parasiticides available to protect dogs and cats from ectoparasites, the market for novel, more effective and more convenient products continue to grow. Isoxazolines are a potent new class of ectoparasiticide for companion animals. These compounds have demonstrated remarkable activity against the most common parasites of dogs, fleas and ticks, via the oral route of administration. This has been a major breakthrough in parasite control, particularly for ticks, as the best previous acaricides have been topically administered products. This new isoxazoline class of parasiticide also has advantages over the first generation of oral parasiticides available to veterinarians, which had limited ability to effectively kill both fleas and ticks on dogs (Beugnet and Franc, 2012). Fast and consistent efficacy for the full dosing period against fleas and multiple species of ticks is an important attribute

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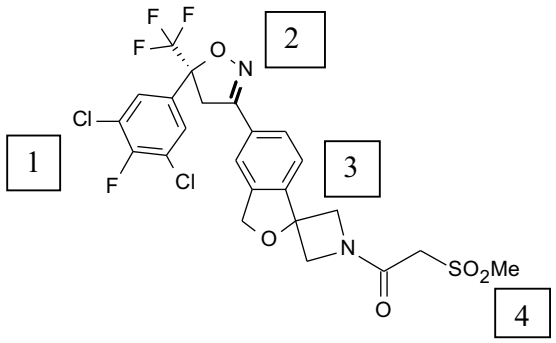


Fig. 1. Structure of sarolaner: 1) phenyl head group, 2) isoxazoline core, 3) spiroazetidinebenzofuran moiety and 4) methylsulfonylethanone tail.

of effective ectoparasiticides, both as a means to improve basic parasite control and to reduce the potential for pathogen transmission. Products with a faster onset of action will help achieve both these aims.

The mechanism of action (MOA) of the isoxazoline class of compounds is well documented. Previous reports from the literature have demonstrated that the isoxazolines exhibit antiparasitic activity through specific blockade of insect GABA- and glutamate-gated chloride channels (Garcia-Reynaga et al., 2013; Gassel et al., 2014; Ozoe et al., 2010). The *in vivo* efficacy of several of these compounds (afoxolaner and fluralaner) have been previously described in numerous publications (Hunter et al., 2014; Rohdich et al., 2014; Shoop et al., 2014; Wengenmayer et al., 2014).

This paper describes the compound attributes, *in vitro* screening strategy, initial *in vivo* ectoparasiticidal activity, pharmacology and mechanism of action (MOA) and basic pharmacokinetic (PK) profile of the novel isoxazoline, sarolaner. Sarolaner was designed in the research laboratories at Zoetis and is the product of an intensive effort to discover and develop a novel isoxazoline specifically for use in companion animals.

2. Materials and methods

2.1. Compound background and details

Sarolaner (1-(5′-((5*S*)-5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)-3′-H-spiro(azetidine-3,1′-(2) benzofuran)-1-yl)-2-(methylsulfonyl) ethanone) was discovered during a 2-year lead optimization program. Over 3000 isoxazoline compounds were prepared and tested to build an understanding of the structure activity relationships of the isoxazoline class that led to the selection of sarolaner as the best molecule for advancement as a candidate for product development. The molecule's structure can be described as four connected subunits; a substituted phenyl ring head group, the isoxazoline core, a spiroazetidinebenzofuran moiety and a methylsulfonylethanone tail (Fig. 1). The molecule was optimized for broad-spectrum ectoparasiticidal potency, pharmacokinetics and safety in the dog as follows. The addition of a 4-substituted fluorine to a 3,5-dichlorophenyl head unit provided superior tick potency compared to any 4-hydrido-3,5-substituted patterns. The isoxazoline was prepared as a single S-enantiomer due to all of the activity residing in this chiral conformation; the R-enantiomer provided no potency against fleas or ticks. Mindful of enhancing safety in canine patients, selecting the chirally pure form minimized potential off-target effects that could result from the incorporation of the inactive enantiomer. The spiroazetidinebenzofuran moiety is a unique structure, previously undescribed in the parasitology literature, providing rigidity, potency and novelty to the molecule. The final piece of optimization, yielded the methylsulfonylethanone tail, increasing the polar surface area of the molecule and maximizing the pharmacokinetic exposure to target the rapid kill of fleas and ticks.

Afoxolaner and fluralaner, which were originally both discovered in the agrochemical field, are presented below for reference (Fig. 2).

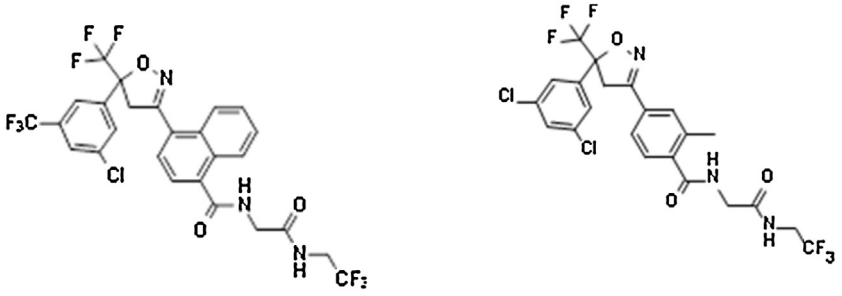
2.2. Evaluation strategy

Preliminary efficacy screening was conducted *in vitro* against the cat flea, *Ctenocephalides felis felis*, with active hits subsequently screened against the soft tick, *Ornithodoros turicata*. Once initial efficacy had been profiled *in vitro*, the safety of selected compounds was assessed in a mouse symptomatology model and those compounds with an acceptable rodent safety profile were progressed to target animal toleration, pharmacokinetics and efficacy studies in the dog.

For all studies involving animals reported in this paper, animals were handled with due regard to their welfare and all protocols and procedures were reviewed by appropriate welfare authorities and applicable procedures were conducted according to state and national/international regulations.

2.3. In vitro *screening—fleas (*Ctenocephalides felis*)*

Fleas were reared using an artificial dog apparatus to provide regular supplies of an established laboratory strain of fleas (*C. felis felis*) not more than 48 h post-emergence. Approximately 30 adult fleas (mixed sex) were collected and aspirated into the feeding chambers (modified 50 mL centrifuge tube with a 300 mm mesh top) and held at 25° C and 75% relative humidity (RH) until use. Test compounds were made up as stock solutions in 0.5% dimethyl



Afoxolaner Fluralaner

Fig. 2. Structures of other isoxazolines.

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